PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Manja Ahola et al.

Serial Number: 10/828,351 Group Art Unit: 1615

Filed: April 21, 2004 Examiner: Tran, Susan T.

For: DISSOLVABLE OXIDES FOR BIOLOGICAL APPLICATIONS

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Mika JOKINEN, hereby declare as follows:

1. I am the Research Director of DelSiTech Ltd., the owner of U.S. Patent Application S.N. 10/828,351 (hereinafter "this application"). My resume is attached.

2. I have read and understood this application, including the claims. Claims 23-31 will be pending upon entry of the attached Amendment.

3. Claims 23-31 are directed to a method of administering a biologically active agent into a human or animal body, wherein said method consists essentially of implanting, injecting, or transmucosally attaching a delivery device, wherein said delivery device comprises a controllably dissolvable silica-xerogel produced by a sol-gel process, and wherein said

silica-xerogel contains a biologically active agent, and controllably releasing said biologically active agent at a substantially constant rate by complete dissolution of said silica-xerogel over a desired time period when in contact with body fluid,

wherein release of the biologically active agent from the silica-xerogel is based on said dissolution.

4. I have read the Official Action dated January 16, 2007, and understand claims 23-28, 31 and 32 stand rejected as obvious over U.S. Patent No. 5,591,453 to Ducheyne et al. in view of International Patent Publication WO 92/20623 to Einarsrud et al.

5. I analyzed the Ducheyne et al. Examples and Figures to determine whether its silica-based matrix releases its active agent primarily by dissolution of the matrix or by diffusion.

6. The power law based on Fick's second law of diffusion is commonly used to analyze the mechanism of release from a matrix: $y = (k)(t)^n$

where

y is the cumulative amount of biologically active material released from the matrix;

k is a sample dependent constant based on structural and geometric characteristics, but which does not affect interpretation of the release mechanism;

t is time; and

n is an exponent.

7. The above equation is valid until 60% of the biologically active agent has been released from the matrix. Except for the small granules data of Fig. 3, none of the Ducheyne et al. examples exceed this 60% maximum release limitation. As for the small granules of Fig. 3, most of their release occurs at the first point of measurement, i.e., an initial burst release from the matrix surface. Fig. 3 does not show or indicate that release from the small granules is controlled by either diffusion through the small granules or dissolution of the granules.

8. The value for the exponent n which results from fitting a release curve to the above equation indicates whether release of the biologically active agent from a matrix occurs primarily by diffusion through the matrix, by matrix dissolution or by a combination of both mechanisms:

when n is from 0.4 to 0.5, release occurs through diffusion of the biologically active agent through the matrix;

when n is from above 0.5 to 1.0, a combination of release mechanisms may be present;

when n is about 1.0, release occurs by matrix dissolution.

9. Release of the biologically active agent is not controlled by either diffusion through the matrix or by dissolution of the matrix when the exponential value is clearly less than 0.4-0.5. Although such low values have not been directly associated with surface release, release of the biologically active agent from only the surface of the matrix and/or from its outermost layers is the most likely explanation.

10. Ducheyne et al.'s examples illustrate the incorporation and release of three biologically active agents (vancomycin, trypsin inhibitor and transforming growth factor TGF-β1) from silica-based matrices. Seven of the Ducheyne et al. figures are release profiles from SiO_2 matrices, and another figure illustrates release from a silica matrix containing calcium and phosphorus.

11. Figure 3 of Ducheyne et al. depicts the release of vancomycin over time from small and large granules of pure

silica glass immersed in a simulated physiological solution, as reported in Example 3. Two silica glass discs having different surface area/volume ratios were also evaluated. Solving for the exponential value n produced the following results:

Large granules (5x5x2 mm) n is 0.66, R^2 is 0.92

silica discs (SA/V of 1.1 mm^{-1}) n is 0.75, R^2 is 0.90

silica discs (SA/V of 0.8 mm^{-1}) n is 0.92, R^2 is 0.95.

The data for the small granules' release is not included because more than 60% of the biologically active agent was released. See paragraph No. 7.

12. The exponential values for the large granules and both silica discs are above 0.5, which indicates a combination of release mechanisms. However, the limited number of data points provided by Ducheyne et al. makes the analysis uncertain, as shown by regression analysis (Perfect curve fitting would produce an R^2 of 1.00). The exponential values listed below were calculated without including the first data point in an attempt to fit the curves more closely:[1]

[1]Exclusion of the first or last data point(s) is justified if there is a clear deviation from the main phase of the release curve (either clearly faster initial phase, also called "burst" that indicates that the biologically active agent is concentrated on or near the surface and is consequently released quickly, or a clearly slower last phase, which commonly indicates that the encapsulated biologically active agent is being depleted or that the matrix will not release anymore agent due to some other reason, e.g., matrix structure, experimental set-up, etc.). However, there are typically more data points than those shown in Fig. 3 before one would decide to exclude the first or last data points.

Large granules	(5x5x2 mm)	n is 0.41, R^2 is 0.94
silica discs	(SA/V of 1.1 mm^{-1})	n is 0.43, R^2 is 0.94
silica discs	(SA/V of 0.8 mm^{-1})	n is 0.67, R^2 is 0.96

13. In a second attempt to fit the curves more accurately, the exponential value n was recalculated by excluding the last data point only (the first data point was included in the calculation):

Large granules	(5x5x2 mm)	n = 0.75, R^2 is 0.94
silica discs	(SA/V of 1.1 mm^{-1})	n = 0.86, R^2 is 0.93
silica discs	(SA/V of 0.8 mm^{-1})	n = 1.04, R^2 is 0.97

14. The data in Paragraph Nos. 11-13 suggest a combination of release mechanisms may be present in Fig. 3, with the results for the silica discs having a SA/V of 0.8 mm^{-1} perhaps more influenced by a matrix dissolution release mechanism than by diffusion of the biologically active agent through the matrix. However, the limited number of data points in Fig. 3 makes these results uncertain, as shown by regression analysis.

15. Figure 4 of Ducheyne et al. depicts the effect of concentration (10 mg vs. 20 mg) on the total amount of vancomycin released over time from SiO_2 discs (11 mm diameter x 2 mm thickness, having a surface area/volume ratio of 1 mm^{-1}).

The release profiles presented in Fig. 4 gave exponential n values of about 0.35, which is close to typical diffusion release values, and far from what would be expected for a matrix dissolution release mechanism:

Sample 1 (10 mg) n is 0.33, $R^2 > 0.99$

Sample 2 (20 mg) n is 0.34, $R^2 > 0.99$

16. The same calculations were performed, with the exception that the first data point was excluded from the curves of Fig. 4:

Sample 1 (10 mg) n is 0.34, $R^2 > 0.99$

Sample 2 (20 mg) n is 0.34, $R^2 > 0.99$

Thus, the curve "fit" remains very close if the first data point is excluded from the calculation.

17. The same calculations were performed, with the exception that the last data point was excluded from the curves of Fig. 4:

Sample 1 (10 mg) n is 0.24, R^2 is 0.84

Sample 2 (20 mg) n is 0.38, R^2 is 0.91

Curve fitting is degraded if the last data point is excluded from the calculation. Nevertheless, all of the exponential n values for Fig. 4 suggest that uncontrolled release from the surface/outer layers of the matrix occurs,

even if a matrix diffusion mechanism may play some role in vancomycin release. Importantly, there is no indication that vancomycin release from the SiO_2 matrix is dependent upon a matrix dissolution mechanism.

18. Figs. 7a and 7b of Ducheyne et al. depict the release of trypsin inhibitor (TI) from granules of a sol-gel derived silica matrix containing calcium and phosphorus through 7 weeks and 4 weeks, respectively. In addition to the difference in release time, Fig. 7a gives the amount released (μg) and in Fig. 7b the concentration (ppm as μg/ml). The exponential values all indicate that release is based upon a diffusion-limited mechanism. There is no indication that release is based on SiO_2 matrix dissolution (where n is about 1.0):

Fig. 7A

TI (10 mg conc.)	n is 0.36, R^2 is 0.996[2]
TI (7.5 mg conc.)	n is 0.47, R^2 is 0.995
TI (5 mg conc.)	n is 0.38, R^2 is 0.995
TI (3 mg conc.)	n is 0.46, R^2 is 0.97
TI (2 mg conc.)	n is 0.46, R^2 is 0.95

[2]Removing the first data point for the 10 mg curve results in an n value of 0.39, with R^2 being 0.997.

Fig. 7B

Protein (10 mg conc.) n is 0.37, R^2 is 0.97

Protein (7.5 mg conc.) n is 0.45, R^2 is 0.98

Protein (5 mg conc.) n is 0.40, R^2 is 0.999

Protein (3 mg conc.) n is 0.49, R^2 is 0.993

Protein (2 mg conc.) n is 0.52, R^2 is 0.98

19. Figure 8 of Ducheyne et al. depicts the effect of the amount, 0.5 µg vs. 1.0 µg, of active transforming growth factor beta (TGF-β1) on its release from pure silica sol-gel glass granules dried to a 50% weight loss, in accordance with Example 9. Calculation of the exponential value n suggests nothing more than an initial burst release of TGF-β1:

1.0 µg TGF-β1 n is 0.23, R^2 is 0.86[3]

0.5 µg TGF-β1 n is 0.14, R^2 is 0.97

20. Fig. 8 provides an inadequate number of data points to conclusively determine the release mechanism(s) present. It should also be noted that the released amounts represent only 0.7-1.1% of the total amount of the encapsulated proteins. The results are for the release of active proteins only, not for all proteins which may have been released (deactivated

[3]Exclusion of the last data point for the 1.0 µg curve resulted in an exponential value n of 0.05, with R^2 being 0.99.

proteins may have also been released). Thus, Fig. 8 does not actually give release results; instead, it is really a determination of the preservation of biological activity by the silica matrix.

21. Figure 9 of Ducheyne et al. compares the effect of drying (50 vs. 70% weight loss) on sol-gel derived silica glass crushed granules loaded with 1.0 µg TGF-β1, in accordance with Example 10.

22. There is an obvious error in the graph of Fig. 9; the symbols have to be reversed to conform with those of Fig. 8. In this case, the released amount active proteins represent only 0.057% of the total amount of protein which has been encapsulated.

23. There are insufficient data points in Fig. 9 to definitely conclude which release mechanism is prevalent. However, the release profiles of Fig. 9 have exponential values which suggest a surface release mechanism is present rather than either a diffusion or a matrix dissolution release mechanism (where n is about 1.0):

50% weight loss n is 0.23, R^2 is 0.86

70% weight loss n is 0.16, R^2 is 0.93[4]

24. Figure 10 of Ducheyne et al. depicts the effect of surface area/volume, granules vs. discs, which have been loaded with 1.0 µg TGF-β1 and dried to a 50% weight loss, in accordance with Example 10. There is an obvious error in the graph of Fig. 10; the symbols have to be reversed to conform with Fig. 8.

25. The released amount of active proteins in Fig. 10 represents only 0.043% of the total amount of protein which has been encapsulated. The exponential n values for the release profiles shown in Fig. 10 suggest release occurs both from surface release and matrix diffusion rather than from matrix dissolution (where n is about 1.0):

50% weight loss granules n is 0.23, R^2 is 0.86

50% weight loss discs n is 0.29, R^2 is 0.96

26. Figure 11 of Ducheyne et al. depicts the release of active TGF-β1, per time period and cumulatively, from discs loaded with TGF-β1 and dried to a 57% weight loss, in accordance with Example 10. The exponential value for the release profiles shown in Fig. 11 (n is 0.38 and R^2 is 0.99)

[4]Exclusion of the last data point results in an exponential n value of 0.12, with R^2 being 0.99.

suggest release by a diffusion mechanism rather than by matrix dissolution (where n is about 1.0).

27. Figure 14 of Ducheyne et al. depicts the release of trypsin inhibitor from sol-gels which contain calcium and phosphorus, in accordance with Example 10. The exponential values n for the release profiles shown in Fig. 14 suggest release by diffusion in every sample, with approximately 9-10% of the total amount of sample encapsulated being released. However, the correlation factor for curve fitting is not very good when all data are included:

2 mg TI n is 0.53, R^2 is 0.82
3 mg TI n is 0.51, R^2 is 0.85
4 mg TI n is 0.49, R^2 is 0.87
5 mg TI n is 0.55, R^2 is 0.89

28. The correlation factors improve (R^2 = to 0.95 to 0.97) for the data in Fig. 14 if the first three data points[5] in each curve is excluded. The exponential values (n = 0.21 to 0.28) suggest fast initial release and some release by diffusion, but also release from the surface and outer layers of the matrix.

[5]Exclusion of the last data point makes the fit even worse than when all data points are included in the calculation.

29. In my opinion, the data in Figs. 8-11 suggest release is controlled by diffusion (Fig. 11) or only from the SiO_2 surface, and that the release illustrated therein is not based on any substantial dissolution of the SiO_2 matrix.

30. Unfortunately, the data presented by Ducheyne et al. do not provide definitive proof of the mechanism(s) governing release of the biologically active agent from the its matrix due to the methodology employed. Ducheyne et al. exchanged the simulated physiological solution during his experiments. The use of fresh solution may have masked the mechanism of release, i.e. diffusion or matrix dissolution. For example, if the solution was quickly saturated by the dissolving SiO_2 matrix due to the small volume of solvent, further SiO_2 dissolution (and release of active agent due to such SiO_2 dissolution) would be halted as soon as saturation was reached. SiO_2 dissolution (and active agent release via a degradation mechanism) might begin again upon replacement of the old saturated solution with fresh solution, until the saturation point was reached again. Thus, results which apparently suggest release from the surface and/or release by diffusion could be the result of mixed mechanisms.

31. Another problem with the Ducheyne et al. data is that it covers only a limited time period during which only a small percentage of biologically active agents is released. When the data covers only 10% or less of the encapsulated biological agent, it is difficult to conclude whether such data is representative of the release mechanism(s).

Conclusions

32. In my opinion, the Ducheyne et al. figures do not provide definite proof of the release mechanism(s) involved.

33. In my opinion, the Ducheyne et al. matrices do not dissolve completely during their release of biologically active agent regardless of the release mechanism(s) involved. In most of the examples only a minor portion of the biologically active agent is released and consequently the major portion remains incorporated in the matrix. Accordingly, the matrix cannot be completely dissolved. In the few examples which indicate substantial release of the biologically active agent the data indicates release by diffusion and/or release from the surface, both of which exclude complete dissolution of the matrix.

34. I further declare all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that further these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent resulting therefrom.

Signed this 18th day of June, 2007.

Signed: _____

Name: Mika Jokinen

Attachment:
Resume of Mika Jokinen